P11

Development of a micronucleus test using the EpiAirway™ organotypic human airway model

T. Hashizume[1]*, S. Munakata[1], T. Takahashi[1], S. Kimuro[1], K. Ishimori[1], & T. Watanabe[1]

[1] Japan Tobacco Inc., Yokohama, Japan
** tsuneo.hashizume@jt.com*

The use of organotypic human tissue models in genotoxicity has increased as an alternative to animal testing. Genotoxicity is generally examined using a battery of in vitro assays such as Ames and micronucleus (MN) tests that cover gene mutations and structural and numerical chromosome aberrations. At the 7th International Workshop on Genotoxicity Testing, working group members agreed that the skin models have reached an advanced stage of maturity, while further efforts in liver and airway models are needed [Pfuhler et al., Mutat. Res. 850-851 (2020) 503135]. Organotypic human airway model is composed of fully differentiated and functional respiratory epithelium. However, because cell proliferation in organotypic airway models is thought to be less active, assessing their MN-inducing potential is an issue, even in the cytokinesis-blocking approach using cytochalasin B (CB) [Wang et al., Environ. Mol. Mutagen. 62 (2021) 306-318]. Here, we developed a MN test using EpiAirway™ in which epidermal growth factor (EGF) was included as a stimulant of cell division.

By incubating EpiAirway™ tissue with medium containing various concentrations of CB, we found that the percentage of binucleated cells (%BNCs) almost plateaued at 3 µg/mL CB for 72 h incubation. Additionally, we confirmed that EGF stimulation with CB incubation produced an additional increase in %BNCs with a peak at 5 ng/mL EGF. Transepithelial electrical resistance measurement and tissue histology revealed that CB incubation caused the reduced barrier integrity and cyst formation in EpiAirway™. Adenylate kinase assay confirmed that the cytotoxicity increased with each day of culture in the CB incubation period with EGF stimulation. These results indicated that chemical treatment should be conducted prior to CB incubation. Under these experimental conditions, it was confirmed that the frequency of micronucleated cells was dose-dependently increased by apical applications of two clastogens, mitomycin C and methyl methanesulfonate, and an aneugen, colchicine, at the subcytotoxic concentrations assessed in %BNCs.

Well-studied genotoxicants demonstrated capability in an organotypic human airway model as a MN test system. For further utilization, investigations of aerosol exposure, repeating exposure protocol, and metabolic activation are required.

Keywords:
Micronucleus test; organotypic human airway model; EGF; clastogens; aneugen.